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STUDIES ON
THE CYTOLOGY OF ARCTIC PLANTS

II

HABENARIA

BY

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WITH 20 FIGURES IN THE TEXT

KØBENHAVN

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BIANCO LUNOS BOGTRYKKERI A/S

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Fig. 1. *Habenaria hyperborea* var. *major* at Kitdlüssat,
31-7-1932.

Under extreme conditions of climate it is no uncommon thing for plants to display special cytological conditions, certain races for example having other chromosome numbers, usually higher, more rarely lower, than the number associated with the species within the range of its normal distribution. For this reason the chromosome numbers of arctic plants are of particular interest. In the present case the author has chosen some of the few arctic representatives of the Orchidaceae family, which of course is centered in the tropics where it has thousands of species, but which decreases rapidly as one moves northwards. While in Greenland in 1932 I therefore fixed material of *Habenaria hyperborea* (L.) R. Br. var. *major* Lge. and *H. straminea* FERNALD (*H. albida* var. *straminea*) in a locality lying close to the northern limit of the species: Kitdlüssat on the south coast of Disko in lat. 69°23' N. The locality was a snow-patch with a rich vegetation of *Equisetum*

variegatum and *E. arvense*, in among which there are more sporadic occurrences of *Habenaria*, *Bartschia*, *Polygonum* and several others (figs. 1 and 10).

This material was supplemented by collections from Iceland of *Habenaria hyperborea* (L.) R. Br. var. *minor* Lge., kindly furnished by Dr. O. HAGERUP (fig. 5).

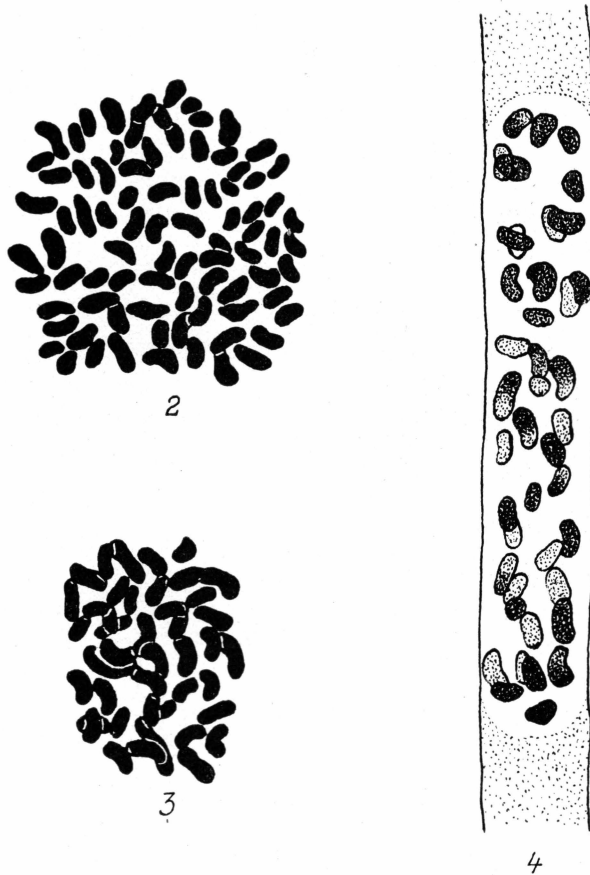


Fig. 2. Metaphase in root-tip, $2n = 84$. $\times 3300$.

Fig. 3. Homotypic metaphase at embryo-sac formation, $n = 42$. $\times 3300$.

Fig. 4. Metaphase of division in pollen tube, $n = 42$. $\times 3300$.

The Icelandic material was fixed in Carnoy's fixative, the Greenland material in Karpechenko's fixative after a brief pre-fixation in Carnoy. In most cases staining was carried out according to Feulgen. Good results were obtained with a hydrolysis time of 6 minutes for the Carnoy-fixed material and 12 minutes for that fixed with Karpechenko's fixative. In both cases the staining time was 45 minutes.

The Herbarium material gave reasons for anticipating polyploidy, there being a considerable difference in size between the two forms of *Habenaria hyperborea*, the southerly-occurring var. *minor* and the northerly var. *major*, of which the latter is the larger and more vigorous. Polyploidy has previously been found in a number of European orchids (HAGERUP, HEUSSER).



Fig. 5. *Habenaria hyperborea* var. *minor* at Reykjavik. 6-7-1931. Nat. size.

Habenaria hyperborea var. *major*.

The chromosomes in the somatic divisions in the root-tips were rather small, short and rod-shaped, and often slightly curved; the number was $2n = 84$ (fig. 2). In the metaphase of the homotypic division in the embryo sac the difference between the individual chromosomes seemed to be greater (fig. 3). There, and also in divisions in the pollen tubes (fig. 4) the number was $n = 42$.

Habenaria hyperborea var. *minor*.

In pollen tubes and in the first division in the pollen grains I found $n = 42$ (figs. 6, 7 and 8). The chromosomes proved to be very different in size, but there was a smooth transition from the largest to the smallest, the largest being about three times as large as the smallest. The same number was observed in divisions at the formation of the

embryo sac, both diakinesis and meiosis phases. In the first metaphase of meiosis (fig. 9) there was again a considerable difference in the size of the chromosomes.

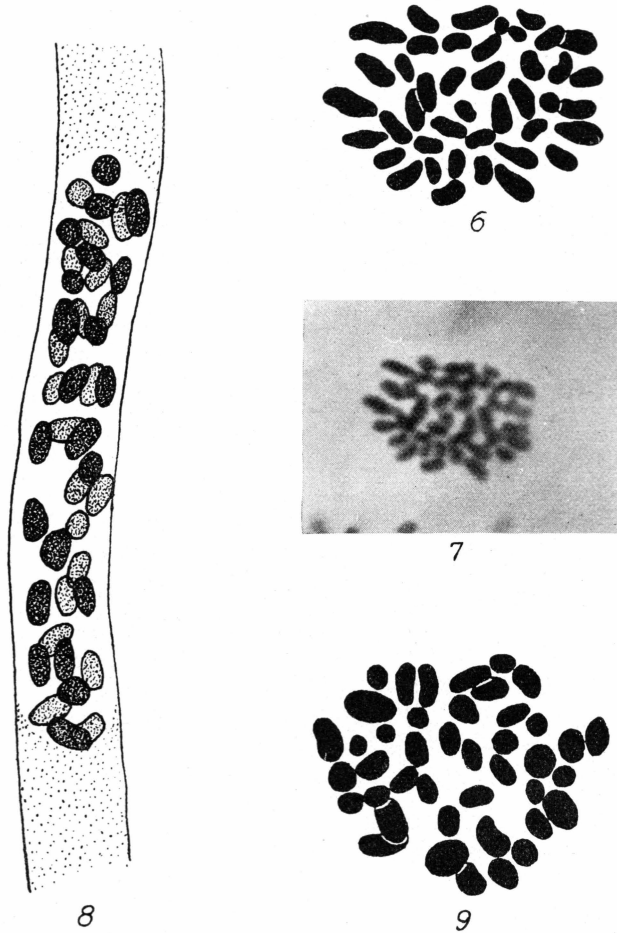


Fig. 6. Metaphase of first division in pollen grain, $n = 42$. $\times 3300$.

Fig. 7. Metaphase of first division in pollen grain, $n = 42$. \times ca. 2000.

Fig. 8. Metaphase of division in pollen tube, $n = 42$. $\times 3300$.

Fig. 9. Heterotypic metaphase at embryo-sac formation, $n = 42$. $\times 3300$.

Habeneria straminea.

In the somatic divisions in root-tips I counted $2n = 42$ (fig. 11). The chromosomes, which were rather small, averaged slightly larger than those of *H. hyperborea*. The largest were almost twice as long as the smallest; they were short, rod-shaped, and mostly a little curved.

In divisions in the pollen tube I counted $n = 21$ (fig. 12). The same was observed in divisions in the embryo sac; the first metaphase of meiosis gave no picture that could be used, whereas there were several clear



Fig. 10. *Habeneria straminea* at Kitdlüssat. 31-7-1932.

plates of the homotypic division (figs. 13 and 14). There the chromosomes were rather slender but otherwise of almost the same size as in the root-tips.

The material was too old to show meiosis when the pollen was formed. But at the first division in the young pollen grains of *H. hyperborea* the two nuclei then formed were distinctly different, the generative nucleus being rather small and spherical and staining very intensely, whereas the vegetative nucleus was large, somewhat long and only faintly stained; in the somewhat older pollen grains it had become almost bowl-shaped, with the concave side turned towards the generative

nucleus (fig. 15). Something similar was observed in *Habenaria albida*, but there the vegetative nucleus in most cases was only flattened on one

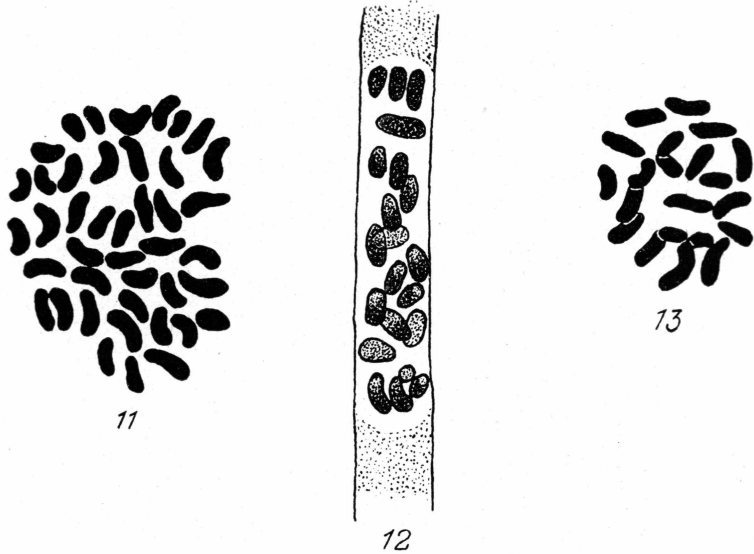


Fig. 11. Metaphase in root-tip, $2n = 42$. $\times 3300$.

Fig. 12. Metaphase of division in pollen tube, $n = 21$. $\times 3300$.

Fig. 13. Homotypic metaphase at embryo-sac formation, $n = 21$. $\times 3300$.

side, and not depressed as on *H. hyperborea* (fig. 16). This difference between the two nuclei as regards size and staining was also observed by HAGERUP in *Orchis*.

Fertilization seems to have a normal process, no sign being observed of self-fertilization or apogamy. Division of the generative

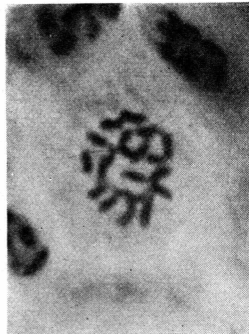


Fig. 14. Homotypic metaphase at embryo-sac formation, $n = 21$. \times ca. 2000.

nucleus proceeds simultaneously with the growth of the pollen tube into the cavity of the ovary. Almost simultaneously meiosis proceeds with

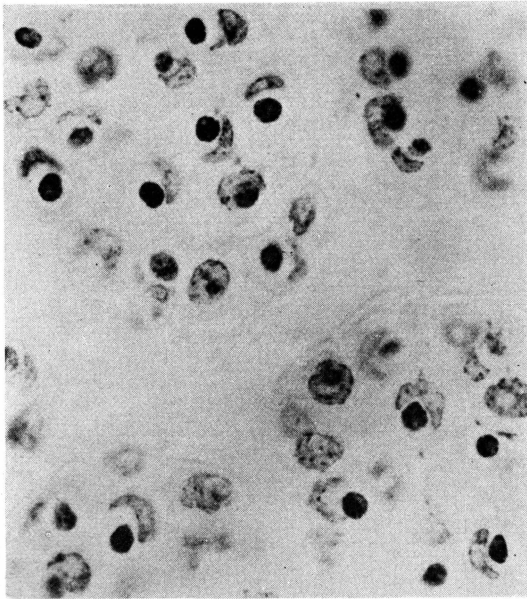


Fig. 15. *Habenaria hyperborea* var. *minor*, young pollen grain; note the difference between the two nuclei in the grain.

the formation of the embryo sac. The oldest parts of the material have pollen tubes right down to the bottom of the ovary, but as yet no fully

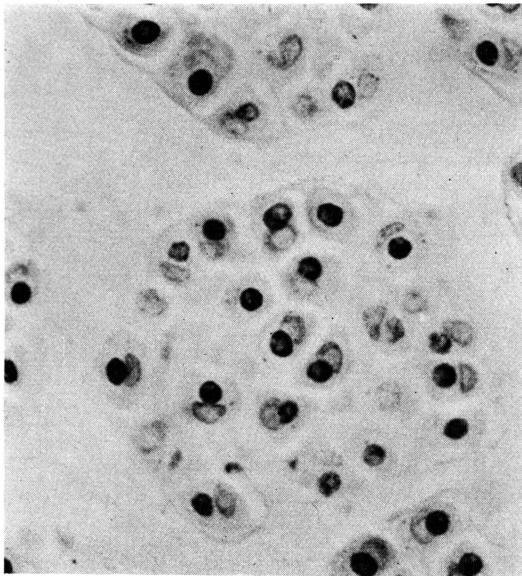


Fig. 16. *Habenaria albida*, young pollen grain.

developed embryo sac. The pollen tubes mostly follow the three rows of ovules in the ovary and within the thicknesses visible on the outer side.

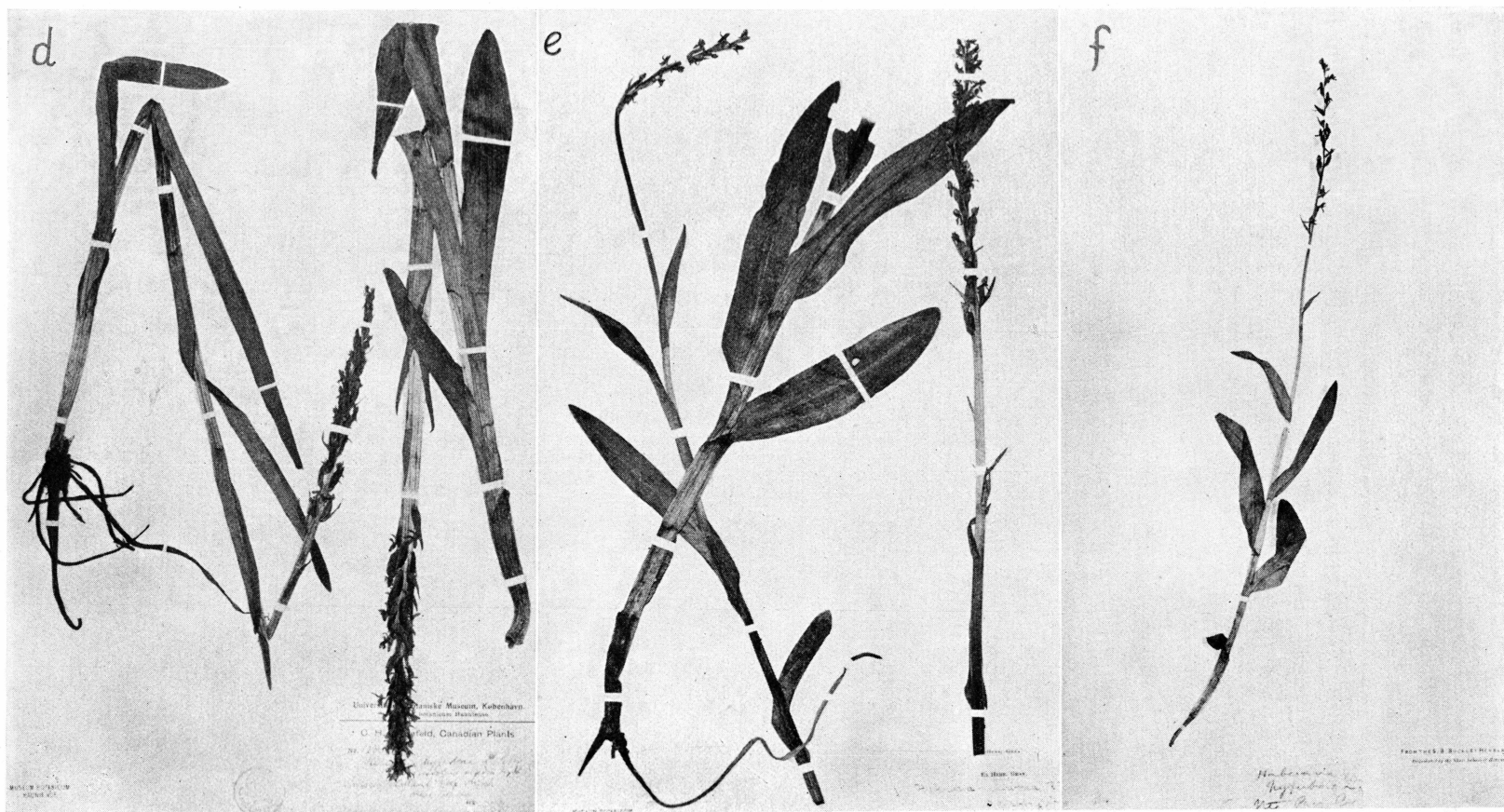


Fig. 17d—f.

Fig. 17. Herbarium material of *Habenaria hyperborea* from North America: a. California, b. Massachusetts, c. Canada (Quebec), d. Canada (Ontario), e. Connecticut, f. Colorado, ca. 1:3.

Habenaria hyperborea has been examined before. HUMPHREY found $2n = 42$ in material from Minnesota, U. S. A. The same number has been observed in a number of other species of the same genus in North America, where it is richly represented. With a single exception (*H. bracteata*) the chromosome picture is the same for all species examined, with rather small, short, rod-shaped and slightly curved chromosomes with no great mutual variation as to length. In the present material

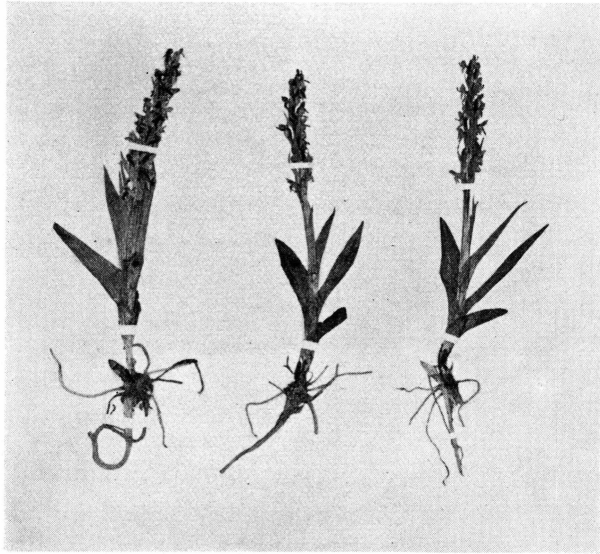


Fig. 18. Herbarium material of *Habenaria hyperborea* (var. *major*) from Kitdlüssat, ca. 1:3.

the largest chromosomes are two or three times the length of the smallest and have smooth transitions between them.

The varieties *major* and *minor* of *H. hyperborea* differ from each other principally by their size only; otherwise the species in Greenland and Iceland seem to be very homogeneous, whereas the American forms differ considerably. Compared with herbarium material from Canada and U. S. A. (fig. 17) they are smaller as a rule, but give the impression of being more compact (fig. 18). Of the material at my disposal the plants most closely approaching these varieties habitually are those from Canada (Ontario, fig. 17d). The collective species ranges from East Asia across North America to Greenland and Iceland.

H. hyperborea is a fresh example of the many species which in arctic regions are represented by tetraploid races. In polyploid races it has often been found that the cells are larger than those of the diploid races. Consequently it should be possible by comparing the size of e. g. the

stomata to obtain an indication of whether an arctic race has an increased chromosome number over against the corresponding non-arctic race. I therefore took measurements of the size of the stomata in herbarium material of plants from U. S. A., Canada, Greenland and Iceland, but the variations were so great from plant to plant that

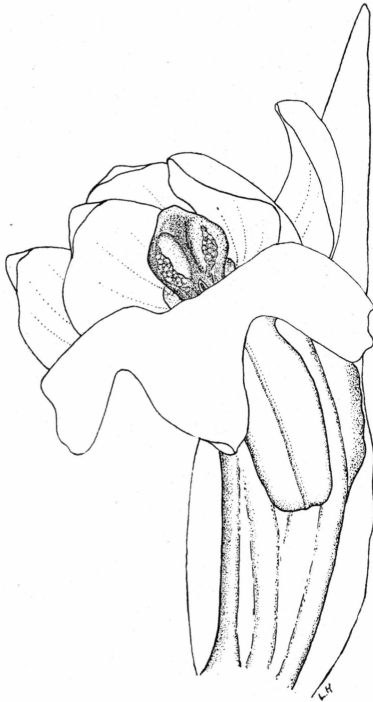


Fig. 19. Flower of *Habenaria straminea*,
× 10.

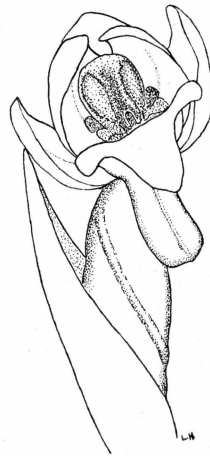


Fig. 20. Flower of *Habenaria albida*,
× 10.

on this basis it was impossible to say anything as to the supposed chromosome number.

Habenaria straminea is closely related to *H. (Gymnadenia) albida* R. Br., which has not so many forms as *H. hyperborea*. It occurs in the north of New Foundland, Greenland, Iceland and the Faeroe Islands, whereas *H. albida* has a wide distribution in Europe. The species was described by FERNALD, and it differs from *H. albida* especially by the longer bracts, which are longer than the flower (figs. 19 and 20), and by the yellowish, almost membranous petals. HEUSSER examined the chromosome number of *H. albida* and found $2n = 42$. The shape and size of the chromosomes agree with the present material of *H. straminea*.

Summary.

The chromosome numbers of *Habenaria hyperborea* from Greenland and Iceland and *H. straminea* have been determined in divisions in root-tips, pollen cells and at the formation of the embryo sac.

In the present arctic material *Habenaria hyperborea* has $n = 42$ and thus is tetraploid, whereas more southerly forms are diploid (HUMPHREY).

H. straminea has $n = 21$.

The chromosomes are fairly homogeneous and the results agree with earlier publications concerning *Habenaria*.

At the first division in the pollen grains the two nuclei thereby formed are different as regards size, shape and stainability.

Divisions in the pollen tube take place when the tube grows into the cavity of the ovary, almost simultaneously with the meiotic divisions at the forming of the embryo sac.

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